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DNA NANOTECHNOLOGY FOR MASSIVE INFORMATION STORAGE

New York University

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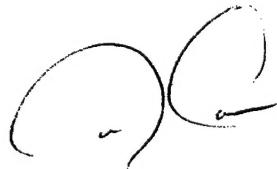
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Studies conducted under the sponsorship of this award consisted of advances in DNA nanotechnology so that it will ultimately be useful for massive information storage. The highlights of the progress fall into the categories of DNA nanomechanical devices, self-assembled DNA arrays in 1D and 2D, and the development of new DNA motifs, and their use in these systems. We have developed a DNA nanomechanical device predicated on the B-Z structural transition of DNA. This rotary device is the first DNA machine, and it has been used to prototype DNA nanomechanical devices in general. Later in the project period we developed a new theory for producing DNA structural motifs, based on reciprocal recombination of DNA double helices. This theory led to a new motif called PX DNA, based on the paramecic association of two double helices. PX DNA, in turn, has been used to produce the first robust rotary DNA nanomechanical device that is based on DNA sequence, rather than environment. In the area of self-assembling arrays, we have had major successes. To begin, we have used rigid DNA double crossover molecules as edges of triangles to produce 1D arrays of well-defined features. In 2D, we learned for the first time to modify 2D arrays so that we can add (or remove) materials to them, materials we expect eventually to aid the assembly of nanoelectronics. We also developed new 2D elements for building arrays, including DNA parallelograms with tunable cavities made both of conventional and bowtie DNA branched junctions. We also developed DNA triple crossover molecules as lattice components. We used them for 2D arrays that offer an avenue to 3D, and for the first algorithmic self-assembly likely to lead to DNA-based computation and self-assembled circuitry.		24	
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SUMMARY

The research sponsored by this contract has focused primarily on the facilitation of extremely dense bottom-up assembly of uniquely placed components. The goal ultimately is to produce units that can contain information in 2D or 3D arrays of extremely small dimension. The most important results of the research are listed below:

1. The development of DNA nanomechanical devices that will be useful in tuning the positions of information-containing components. These include both a device based on the B-Z (right-handed to left-handed) transition of DNA, and a device that is sequence based. The latter entails a new DNA motif based on a new principle of motif generation. Its testing relied on a fixed array (not a device) developed from 1D DNA triangle arrays.
2. The development of 2D arrays from DNA parallelograms based on conventional and bowtie DNA branched junctions. These are elements that contain tunable cavities that are likely to be useful in localizing nanoelectronic components. The bowtie junction is a DNA branched junction with unusual polarity in two strands.
3. The development of the capability of modifying 2D arrays, so that new materials can be added to them. Thus, any chemical species that can contain or transmit information can be added to an array after its formation.
4. The development of triple crossover molecules has led to further advances. These molecules represent a natural way to extend 2D self-assembled arrays to 3D arrays, thus increasing the density of information in a truly dramatic fashion.
5. The use of DNA triple crossover molecules in an algorithmic assembly is the first non-periodic self-assembly. A cumulative XOR function was computed by self-assembly, leading naturally to circuit assembly by this method.

INTRODUCTION

The purpose of this contract was to facilitate the storage of information at very high spatial densities. The approach taken is bottom up self-assembly of DNA motifs, which can lead to 1D, 2D, and, ultimately, 3D arrangements of matter that can contain information. We have emphasized development of the expertise to do this by several avenues. We have developed DNA-based nanomechanical devices, whose states can represent information. We have developed parallelogram motifs with tunable cavities that can contain informational components. We have developed the ability to modify 2D arrays, so that DNA carrying other components, such as nanoelectronic components, can be bound to pre-existing arrays. We have developed triple crossover motifs and demonstrated that they can be used to extend 2D arrays to a robust third dimension. We have used triple crossover molecules to perform the first algorithmic self-assembly, computing a cumulative XOR of four steps. This work has resulted in 20 publications and a submitted manuscript, which have cited this contract. These studies will be described below.

METHODS

The methods used in this work are those of DNA Nanotechnology (1). These entail the use of unusual DNA motifs based on systems branched at the secondary structure level, analogous to the Holliday junction of genetic recombination. A prominent motif used is the double crossover (DX) molecule (2), in which two antiparallel double helices are fused together twice as a consequence of strand topology. During the course of this work, earlier characterization of the DX molecule was extended by FRET analysis performed by our subcontractors, Nantronics/Nanogen. A useful extension of the double crossover molecule is the triple crossover (TX) molecule (3), containing three fused helices, whose axes are roughly coplanar. DNA parallelograms usually consist of four conventional 4-arm branched junctions (4). Parallelograms can also be constructed from Bowtie junctions, which contain 3', 3' and 5', 5' linkages in their crossover strands (5); Bowtie parallelograms have the opposite signs of their angles from conventional parallelograms. Paranemic crossover (PX) DNA is similar to DX DNA, except the helices are parallel and fused to each other at all possible positions (6). These motifs are readily derived by a simple topological method we have developed (6).

The branched motifs described above are one component of DNA nanotechnology. The other component is sticky-ended cohesion and/or ligation of DNA molecules. DNA sticky ends have been used for over 25 years by biotechnologists to induce associations between particular DNA molecules, followed by ligation to create cloned genes (7). It is evident to all that the single-stranded overhangs of sticky ends can be used to direct affinity between molecules whose sticky

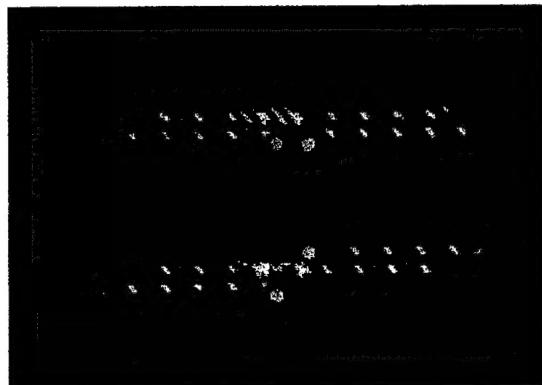
ends are complementary. It is less well-known that sticky-ends cohere to form classical B-DNA in the vicinity of the join (8). Hence, not only is affinity predictably in this system, but so is local product structure. This is important if information is to be stored, because geometry is predictable, not merely topology.

RESULTS AND DISCUSSION

DNA Nanomechanical Devices.

The rigidity of the double crossover unit (2) has permitted us to fulfill an old ambition of ours, to produce a DNA nanomechanical device (9). This one is predicated on the B-Z transition of DNA, and it can be cycled. Its operation has been shown by FRET analysis, and it responds to the presence of $\text{Co}(\text{NH}_3)_6^{3+}$ in solution. The motion, shown in Figure 1 is largely rotatory, leading to motions of 20-60 Å, depending on the distance of the point from the rotation axis.

Figure 1

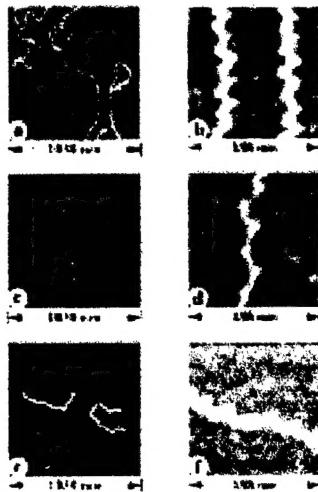


The strength of this system is that it is a robust nanomechanical device. All molecules are in the same state, and the double crossover motif shown maintains its structure well. The weakness of the system is that it is controlled by a small-molecule effector $\text{Co}(\text{NH}_3)_6^{3+}$, so that all copies of the device will be in the same state, depending on its presence. A more informational type of device would be controlled by sequence. Such a device will be described below.

So as to develop a system whereby a sequence-based device could be demonstrated, we built a 1D triangular system, in which triangles with a double crossover molecule for one edge (10). We were able to make demonstrate its effectiveness with the use of the atomic force microscope (AFM). This array is shown in Figure 2. Its zig-zag features are separated by about 32 nm (9 helical turns) in the upper panels, 60.5 nm in the middle panel and 32 nm (all on one side) in the

lower panel. These are all the predicted separations, indicating that we have made a programmable pattern in one dimension.

Figure 2



The type of device that is more useful for information storage or for the development of a nanorobotics must be sequence dependent, but must also remain robust. We have developed such a DNA nanomechanical device. Figure 3 shows the device and its principles of operation.

Figure 3

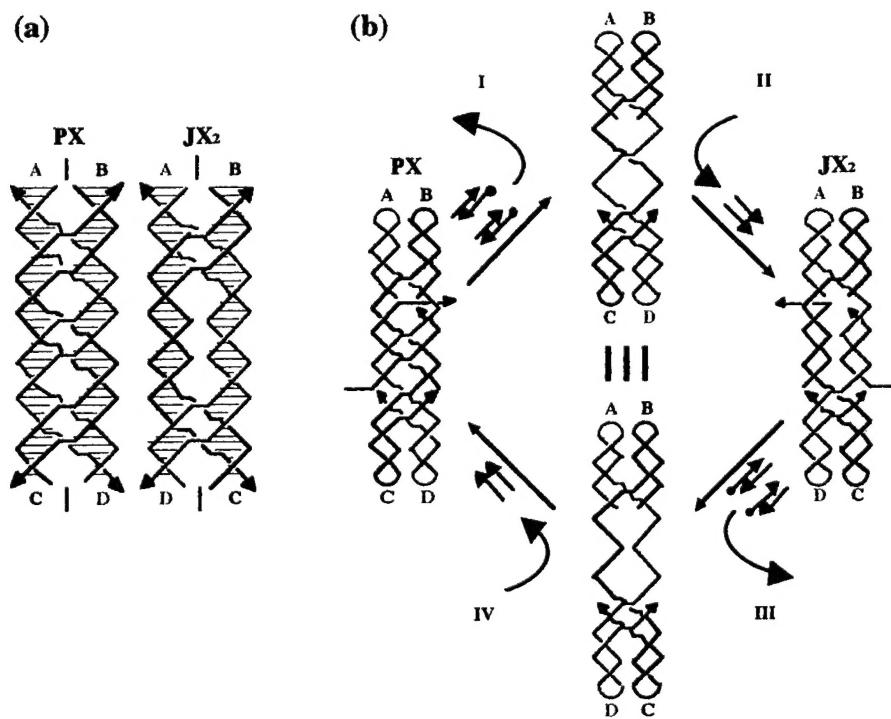
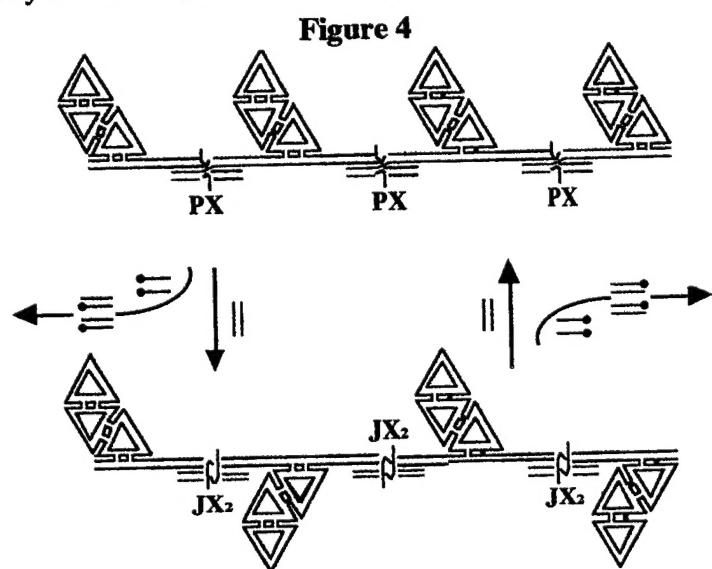


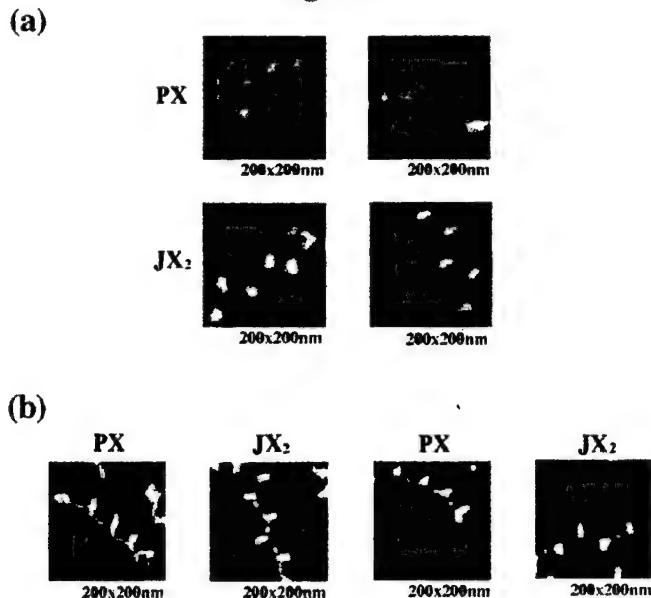
Figure 3a illustrates a PX molecule, and a topoisomer related to it called JX₂, because it is lacking two crossovers between the two helices. Note that the tops of the two molecules, indicated as A and B, are the same in both cases, but that the bottoms are reversed, C and D for PX, but D and C for JX₂. In Figure 3b, the two molecules have been modified. The two red and blue strands of each molecule have been joined into a single strand by the addition of hairpins. In addition, both the red strands and the blue strands have been interrupted by three half-turns of a green (PX) or purple (JX₂) strand. The green and purple strands also contain an extension, so that they can be removed in the presence of a biotinylated complete complement. The operation of the device illustrated in Figure 3b proceeds from PX to the removal of the green strands (process I) to produce an unstructured intermediate. The intermediate is converted to the JX₂ molecule by the addition of the purple strands (process II). The molecule is now in the opposite state. Process III, similar to process I consists of removing the purple strands to produce the same unstructured intermediate (although it is drawn differently for clarity). The cycle is completed by process IV, which restores the PX state by adding the green strands. It is important to recognize that many different species of devices and their associated green or purple strands can be produced, so that an array containing N of them could assume 2^N different structural states.

To demonstrate that the device works, we have shown by gel that the PX and JX₂ molecules produced are robust, lacking either multimers or dissociation products. We have demonstrated by AFM that the device works cleanly. We have used a variant of the triangular 1D array (10) to do this. For ease of visualization, we have replaced the triangles with fused-triangle half-hexagons, connected by the device, as shown in Figure 4.



In the PX state, the molecule is in 'cis' conformation, but in the JX₂ state, it is in a 'trans' conformation. The data demonstrating the efficacy of the device are illustrated in Figure 5.

Figure 5

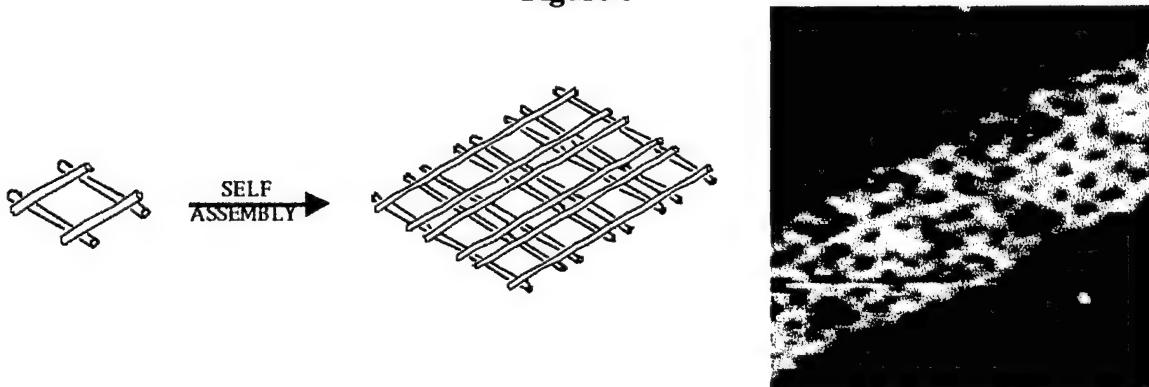


Panel 5a illustrates two control examples for the appearance of the PX and JX₂ conformations in systems like those of Figure 4, except that they are connected by fixed PX and JX₂ molecules. By contrast, panel 5b illustrates 3 steps of operation of the device itself.

DNA Parallelograms

Individual Holliday junctions are floppy, but combining four of them into a parallelogram results in a unit rigid enough to use for an array. The use of Holliday (4) and Bowtie (5) junction arrays allows us to make 2D arrays with defined cavities, as shown in Figure 6.

Figure 6

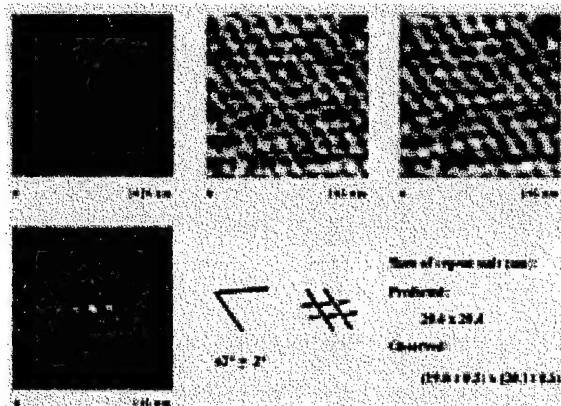


The schematic shows that a repeating array of parallelograms leads to an arrangement of four different parallelograms. The largest one is the basic parallelogram, and the other result from the

overhangs; there are usually not visible when the conventional overhang length of 1 turn (3.4 nm) is used. The largest parallelogram of the array whose AFM is illustrated in Figure 6 has edges of 13 (4 turns) by 20 (6 turns) nm.

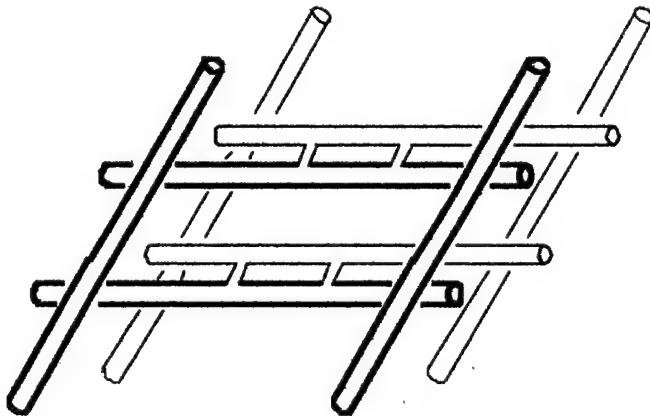
We have established that bowtie parallelograms have the opposite angle (5,11) from conventional parallelograms, as illustrated in Figure 7.

Figure 7



The combination of the two different types of parallelogram motifs suggests a way of approaching 3D construction, as illustrated in Figure 8, wherein a red-blue conventional DNA parallelogram is fused to a green-cyan bowtie parallelogram (11).

Figure 8



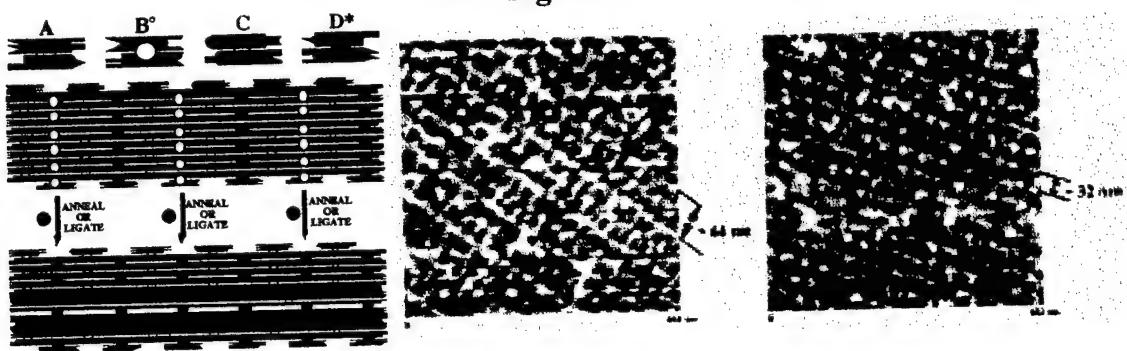
Array Modification

A major success of our program is the ability to produce 2D arrays of DX and TX molecules, in addition to DNA parallelograms. To produce patterns with the DX and TX molecules, we can attach a DNA hairpin that points out of plane of the array, and this serves as a topographic signal

for the AFM. An important achievement under this contract is that we have demonstrated that we can modify the DX arrays, to change the pattern by adding or removing a hairpin (12). Figure 9 shows addition to an array. The colored panel is a schematic showing four tiles, **A**, **B°**, **C** and **D***. **A** and **C** are conventional DX molecules, **D*** has a hairpin, and **B°** has a sticky end, but no hairpin. Sticky ends that hold the array together are represented as complementary geometrical shapes. Addition of a hairpin complementary to the sticky end on **B°** results in a new pattern. Each DX molecule is 16 nm wide x 4 nm high. The left AFM image shows the array before annealing the hairpin, and the prominent stripes correspond to the **D*** hairpins. The sticky ends on **B°** are seen as a weak stripe half-way between the prominent stripes. The right image shows the array after annealing, with the 32 nm stripe.

The ability to modify arrays suggests the possibility that the modifying strands used here can act as carriers for other materials, particularly those involved in nanoelectronics and information storage. We are working currently with a number of groups to use the added hairpin as a carrier to organize other species on the array. These include carbon nanotubes, gold nanoparticles, dyes and magnetic nanoparticles.

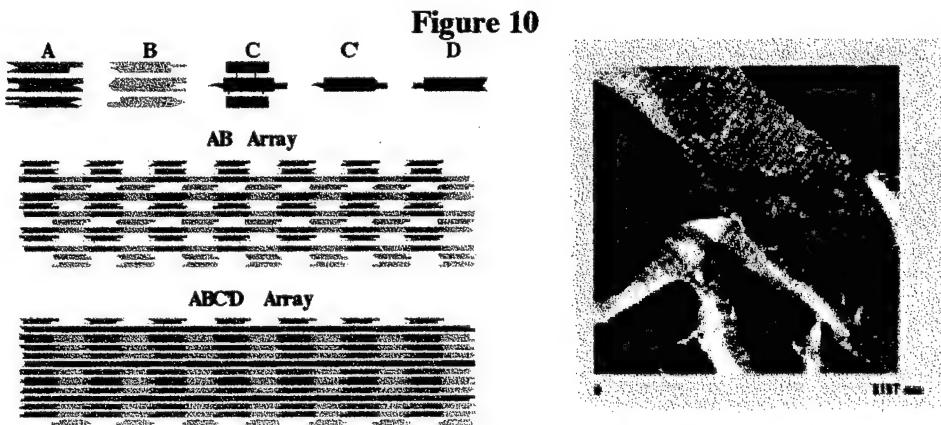
Figure 9



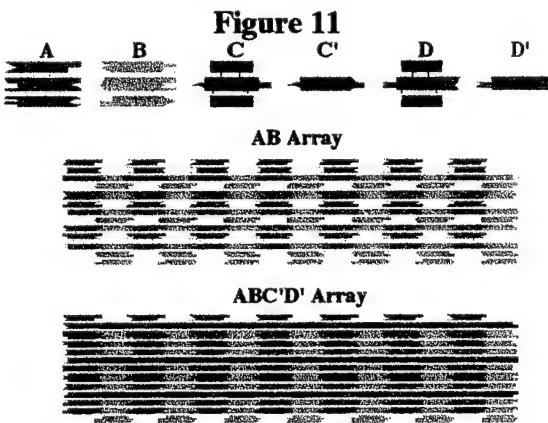
Triple Crossover Molecules

TX molecules (3) enable us to include a gap in the array. There are many reasons why one would want to include a gap in an array, but it should be evident that a gap permits the addition of hetero-materials in a convenient fashion. We are thinking here of including molecules that facilitate the storage and retrieval of information, but in the past gaps have been suggested for the binding of proteins to solve crystallization problems (13). Here, we have prototyped the addition of material to a gap by inserting a helix and a TX molecule into a single-helix gap. This is illustrated in Figure 10, where **A** and **B** represent triple crossover molecules, complementary between their first and third helices, to produce a gap. **D** represents a single helix, which it is easy to imagine inserting into such a gap. **C** represents a triple helix that is also inserted into the

gap; this can be accomplished if the TX molecule has been rotated by three nucleotide pairs (103°). This approach extends the 2D system to 3D in a robust fashion; this is one system we are using to go to 3D. A schematic (in which C is rotated to C') and an AFM are shown in Figure 10.



One important feature of this system is that it suggests a robust route to 3D. Changing D to TX molecule rotated to become D' makes this into a completely 3D system, as illustrated in Figure 11.

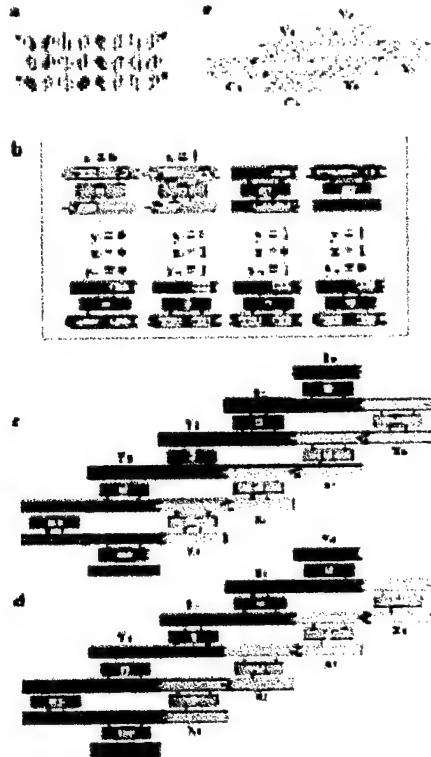


Algorithmic Assembly.

The arrangement of DNA molecules in the arrays of the preceding three sections follow the simplest imaginable algorithm: Periodicity. However, it is possible through self-assembly to program more complex arrangements. The importance of these arrangements is that the expense of producing patterns and circuitry will be decreased vastly if complexity can be programmed, rather than inserted step-by-step. For example, a Sierpinski triangle (Pascal's triangle, mod 2, so that all the even numbers are 0's and all the odd numbers are 1's) can be programmed to yield a fractal pattern with only seven tiles. We have performed cumulative XOR calculations as

prototypes of algorithmic assembly. This is the first time that this type of assembly has been accomplished (14). The system is shown in Figure 12.

Figure 12



The components consist of TX molecules, such as the one shown in panel 12a. Note the red strand, which will act to report the answer at the end. The XOR (exclusive OR) operation produces a Boolean output based on two Boolean inputs. The output is 0 if the two inputs are the same (0 and 0 or 1 and 1), and the output is 1 if the inputs are different (1 and 0 or 0 and 1). In the cumulative XOR operation, $Y_i = \text{XOR}(X_i, Y_{i-1})$, where X_i is the input on the i 'th step and Y_{i-1} is the answer of the last step. Although a general molecular computational approach would perform many calculations in parallel, our experiment was a prototype, in which we did individual calculations, to make sure we could determine the right answer. Tiles (TX molecules) are given values by whether they possess one particular restriction site or another. The blue tiles in panel 12b represent the input X_i tiles, and we assembled them in a prespecified order, 1,1,1, 0 (whose result should be 1, 0, 1, 1) in panel 12 c and 1, 0, 1, 0 (whose result should be 1, 1, 0, 0) in panel 12d. The green tiles serve to initialize the calculation, and the red tiles represent the four possible inputs on their bottom domains, and they transmit their values through their top domains. Thus, the self-assemblies shown in panels 12 c and 12d represent the calculations. To extract an answer from them, the red reporter strands are ligated together, thereby connecting the input with the output. They are then treated with the restriction endonuclease representing 1 or 0 and analyzed on a gel, similar to DNA sequencing.

The results indicate that the calculations have been performed successfully. This is harder for an algorithmic assembly than for a conventional assembly. Whereas in a conventional assembly correct tiles are competing with incorrect tiles for any particular slot, in an algorithmic assembly, the competition is between correct tiles and partially correct tiles: For example, $X_i = 1$ is represented by the same sticky end, regardless of whether it is on a tile representing $X_i = 1$ and $Y_{i-1} = 1$ or $X_i = 1$ and $Y_{i-1} = 0$.

CONCLUSIONS

DNA nanotechnology has been used to develop nanomechanical devices on an extremely small scale, with demonstrated motions ranging from 2-34 nm. By developing sequence-dependent devices, it is likely that information can be stored in the mechanical states of these systems, as well as demonstrating the start of a nanorobotics.

New DNA parallelogram motifs have been developed with small dimensions capable of containing nanoelectronic components within their tunably-sized cavities. Parallelograms of both chiralities are possible through the use of Bowtie junctions.

The development of triple crossover molecules leads naturally to extending the existing 2D systems into 3D. This development is likely to lead to an authentic form of 3D information storage.

The new ability to modify pre-existing DNA arrays suggests that it will be possible to add nanoelectronic components to arrays, using the DNA additions as carriers of those molecules, which are best suited to electronic roles.

The demonstration of algorithmic assembly indicates that for relatively small expense molecular patterns of a complex nature can be produced. Such patterns can scaffold nanoelectronic circuitry.

RECOMMENDATION

The successes of the project described here indicate that DNA nanotechnology should be supported in as many laboratories and to the greatest extent possible. Although it is a long way from immediate application, DNA nanotechnology represents the best way of gaining control of

the structure of matter on the scale that life uses, the scale of a few nanometers. This scale appears to be the practical limit for the foreseeable future, and will endow those who control it with the greatest capabilities for practical technology.

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